

- (c) screening progeny seeds from the fertile plants of step (b) for the desired levels of [unsaturated] hydroxylated fatty acids; and
- (d) processing the progeny seed of step (c) to obtain seed oil containing altered levels of [unsaturated] hydroxylated fatty acids.

15. (amended) A method for altering fatty acids composition in seeds comprising:

(a) making a cross between a mutant line with altered fatty acid composition with a plant [containing] comprising [the] a chimeric gene [of Claim 7] capable of causing altered levels of fatty acids in a transformed plant cell, said chimeric gene comprising a nucleic acid fragment selected from the group consisting of:

(i) an isolated nucleic acid fragment comprising a nucleic acid sequence encoding a plant delta-12 desaturase or a delta-12 hydroxylase wherein said isolated nucleic acid fragment hybridizes to one of the nucleotide sequences set forth in SEQ ID NOS:1, 3, 5, 7, 9, 11, and 15 under one of the following sets of conditions:

(a) hybridization in 50 mM Tris, pH 7.6, 6X SSC, 5X Denhardt's, 0.5% sodium dodecyl sulfate (SDS), 100 µg denatured calf thymus DNA and 50 °C and wash twice with 2X SSC, 0.5% SDS at room temperature for 15 min each, then wash twice with 0.2X SSC, 0.5% SDS at room temperature for 15 min each and then wash twice with 0.2X SSC, 0.5% SDS at 50 °C for 15 min each;

(b) hybridization in 6X SSPE, 5X Denhardt's solution, 0.5% sodium dodecyl sulfate (SDS), 5% dextran sulfate, 100 µg denatured calf thymus DNA at 50 °C and wash twice with 2X SSC, 0.5% SDS at room temperature for 15 min each, then wash twice with 0.2X SSC, 0.5% SDS at room temperature for 15 min each and then wash twice with 0.2X SSC, 0.5% SDS at 50 °C for 15 min each; or

(c) hybridization in 50% formamide, 5X SSPE, 1% sodium dodecyl sulfate (SDS), 1% Denhardt's Reagent, 100 µg denatured salmon sperm DNA at 42 °C and wash twice with 2X SSPE, 0.2% SDS at 42° C for 15 min each, then wash twice with 0.2X SSPE, 0.2% SDS at 55 °C for 30 min each;

(ii) the isolated nucleic acid fragment of (i) wherein the encoded polypeptide has an amino acid identity of 60% or greater to the polypeptide encoded by any one of the sequences set forth in SEQ ID NOS:1, 3, 5, 7, 9, 11, or 15; or

(iii) the isolated nucleic acid fragment of (i) wherein the nucleic acid identity is 90% or greater to any one of the sequences set forth in SEQ ID NOS:1, 3, 5, 7, 9, 11, or 15;

- (b) growing fertile plants from seeds obtained from the cross; and
- (c) screening progeny seeds from the fertile plants of step (b) for seeds containing altered fatty acids.

16. (amended) A method for reducing polyunsaturated fatty acids in rapeseed oil comprising:

(a) making a cross between a rapeseed variety with increased oleic acid content or reduced linolenic acid content with a plant [containing] comprising [the] a chimeric gene [of Claim 7] capable of causing altered levels of fatty acids in a transformed plant cell, said chimeric gene comprising a nucleic acid fragment selected from the group consisting of:

(i) an isolated nucleic acid fragment comprising a nucleic acid sequence encoding a plant a delta-12 desaturase or a delta-12 hydroxylase wherein said isolated nucleic acid fragment hybridizes to one of the nucleotide sequences set forth in SEQ ID NOS:1, 3, 5, 7, 9, 11, and 15 under one of the following sets of conditions:

(a) hybridization in 50 mM Tris, pH 7.6, 6X SSC, 5X Denhardt's, 0.5% sodium dodecyl sulfate (SDS), 100 µg denatured calf thymus DNA and 50°C and wash twice with 2X SSC, 0.5% SDS at room temperature for 15 min each, then wash twice with 0.2X SSC, 0.5% SDS at room temperature for 15 min each and then wash twice with 0.2X SSC, 0.5% SDS at 50 °C for 15 min each;

(b) hybridization in 6X SSPE, 5X Denhardt's solution, 0.5% sodium dodecyl sulfate (SDS), 5% dextran sulfate, 100 µg denatured calf thymus DNA at 50°C and wash twice with 2X SSC, 0.5% SDS at room temperature for 15 min each, then wash twice with 0.2X SSC, 0.5% SDS at room temperature for 15 min each and then wash twice with 0.2X SSC, 0.5% SDS at 50 °C for 15 min each; or

(c) hybridization in 50% formamide, 5X SSPE, 1% sodium dodecyl sulfate (SDS), 1% Denhardt's Reagent, 100 µg denatured salmon sperm DNA at 42 °C and wash twice with 2X SSPE, 0.2% SDS at 42° C for 15 min each, then wash twice with 0.2X SSPE, 0.2% SDS at 55 °C for 30 min each;

(ii) the isolated nucleic acid fragment of (i) wherein the encoded polypeptide has an amino acid identity of 60% or greater to the polypeptide encoded by any one of the sequences set forth in SEQ ID NOS:1, 3, 5, 7, 9, 11, or 15; or

(iii) the isolated nucleic acid fragment of (i) wherein the nucleic acid identity is 90% or greater to any one of the sequences set forth in SEQ ID NOS:1, 3, 5, 7, 9, 11, or 15;

(b) growing fertile plants from seeds obtained from the cross; and

(c) screening progeny seeds from the fertile plants of step (b) for seeds containing reduced polyunsaturated fatty acids.

18. (amended) A method for reducing saturated fatty acids in rapeseed seeds comprising:

(a) making a cross between a rapeseed variety with increased oleic acid content with a plant [containing] comprising [the] a chimeric gene [of Claim 7] capable of causing altered levels of fatty acids in a transformed plant cell, said chimeric gene comprising a nucleic acid fragment selected from the group consisting of:

(i) an isolated nucleic acid fragment comprising a nucleic acid sequence encoding a delta-12 desaturase or a delta-12 hydroxylase wherein said nucleic acid fragment hybridizes to one of the nucleotide sequences set forth in SEQ ID NOS:1, 3, 5, 7, 9, 11, and 15 under one of the following sets of conditions:

(a) hybridization in 50 mM Tris, pH 7.6, 6X SSC, 5X Denhardt's, 0.5% sodium dodecyl sulfate (SDS), 100 µg denatured calf thymus DNA and 50°C and wash twice with 2X SSC, 0.5% SDS at room temperature for 15 min each, then wash twice with 0.2X SSC, 0.5% SDS at room temperature for 15 min each and then wash twice with 0.2X SSC, 0.5% SDS at 50 °C for 15 min each;

(b) hybridization in 6X SSPE, 5X Denhardt's solution, 0.5% sodium dodecyl sulfate (SDS), 5% dextran sulfate, 100 µg denatured calf thymus DNA at 50°C and wash twice with 2X SSC, 0.5% SDS at room temperature for 15 min each, then wash twice with 0.2X SSC, 0.5% SDS at room temperature for 15 min each and then wash twice with 0.2X SSC, 0.5% SDS at 50 °C for 15 min each; or

(c) hybridization in 50% formamide, 5X SSPE, 1% sodium dodecyl sulfate (SDS), 1% Denhardt's Reagent, 100 µg denatured salmon sperm DNA at 42 °C and wash twice with 2X SSPE, 0.2% SDS at 42° C for 15 min each, then wash twice with 0.2X SSPE, 0.2% SDS at 55 °C for 30 min each;

(ii) the isolated nucleic acid fragment of (i) wherein the encoded polypeptide has an amino acid identity of 60% or greater to the polypeptide encoded by any one of the sequences set forth in SEQ ID NOS:1, 3, 5, 7, 9, 11, or 15; or

(iii) the isolated nucleic acid fragment of (i) wherein the nucleic acid identity is 90% or greater to any one of the sequences set forth in SEQ ID NOS:1, 3, 5, 7, 9, 11, or 15;

(b) growing fertile plants from seeds obtained from the cross; and

(c) screening progeny seeds from the fertile plants of step (b) for seeds containing reduced saturated fatty acids.

19. (amended) A method for reducing polyunsaturated fatty acids in soybean oil comprising:

(a) making a cross between a soybean variety with increased oleic acid content or reduced linolenic acid content with a plant [containing] comprising [the] a chimeric gene [of Claim 7] capable of causing altered levels of fatty acids in a

transformed plant cell, said chimeric gene comprising a nucleic acid fragment selected from the group consisting of:

(i) an isolated nucleic acid fragment comprising a nucleic acid sequence encoding a plant a delta-12 desaturase or a delta-12 hydroxylase wherein said isolated nucleic acid fragment hybridizes to one of the nucleotide sequences set forth in SEQ ID NOS:1, 3, 5, 7, 9, 11, and 15 under one of the following sets of conditions:

(a) hybridization in 50 mM Tris, pH 7.6, 6X SSC, 5X Denhardt's, 0.5% sodium dodecyl sulfate (SDS), 100 µg denatured calf thymus DNA and 50 °C and wash twice with 2X SSC, 0.5% SDS at room temperature for 15 min each, then wash twice with 0.2X SSC, 0.5% SDS at room temperature for 15 min each and then wash twice with 0.2X SSC, 0.5% SDS at 50 °C for 15 min each;

(b) hybridization in 6X SSPE, 5X Denhardt's solution, 0.5% sodium dodecyl sulfate (SDS), 5% dextran sulfate, 100 µg denatured calf thymus DNA at 50 °C and wash twice with 2X SSC, 0.5% SDS at room temperature for 15 min each, then wash twice with 0.2X SSC, 0.5% SDS at room temperature for 15 min each and then wash twice with 0.2X SSC, 0.5% SDS at 50 °C for 15 min each; or

(c) hybridization in 50% formamide, 5X SSPE, 1% sodium dodecyl sulfate (SDS), 1% Denhardt's Reagent, 100 µg denatured salmon sperm DNA at 42 °C and wash twice with 2X SSPE, 0.2% SDS at 42° C for 15 min each, then wash twice with 0.2X SSPE, 0.2% SDS at 55 °C for 30 min each;

(ii) the isolated nucleic acid fragment of (i) wherein the encoded polypeptide has an amino acid identity of 60% or greater to the polypeptide encoded by any one of the sequences set forth in SEQ ID NOS:1, 3, 5, 7, 9, 11, or 15; or

(iii) the isolated nucleic acid fragment of (i) wherein the nucleic acid identity is 90% or greater to any one of the sequences set forth in SEQ ID NOS:1, 3, 5, 7, 9, 11, or 15;

(b) growing fertile plants from seeds obtained from the cross; and

(c) screening progeny seeds from the fertile plants of step (b) for seeds containing reduced polyunsaturated fatty acids.

20. (amended) A method for reducing saturated fatty acids in soybean seeds comprising.

(a) making a cross between a soybean variety with increased oleic acid content with a plant [containing] comprising [the] a chimeric gene [of Claim 7] capable of causing altered levels of fatty acids in a transformed plant cell, said chimeric gene comprising a nucleic acid fragment selected from the group consisting of:

(i) an isolated nucleic acid fragment comprising a nucleic acid sequence encoding a plant enzyme encoding a delta-12 desaturase or a delta-12 hydroxylase wherein said nucleic acid fragment hybridizes to one of the nucleotide sequences set forth in SEQ ID NOS:1, 3, 5, 7, 9, 11, and 15 under one of the following sets of conditions:

(a) hybridization in 50 mM Tris, pH 7.6, 6X SSC, 5X Denhardt's, 0.5% sodium dodecyl sulfate (SDS), 100 µg denatured calf thymus DNA and 50°C and wash twice with 2X SSC, 0.5% SDS at room temperature for 15 min each, then wash twice with 0.2X SSC, 0.5% SDS at room temperature for 15 min each and then wash twice with 0.2X SSC, 0.5% SDS at 50 °C for 15 min each;

(b) hybridization in 6X SSPE, 5X Denhardt's solution, 0.5% sodium dodecyl sulfate (SDS), 5% dextran sulfate, 100 µg denatured calf thymus DNA at 50°C and wash twice with 2X SSC, 0.5% SDS at room temperature for 15 min each, then wash twice with 0.2X SSC, 0.5% SDS at room temperature for 15 min each and then wash twice with 0.2X SSC, 0.5% SDS at 50 °C for 15 min each; or

(c) hybridization in 50% formamide, 5X SSPE, 1% sodium dodecyl sulfate (SDS), 1% Denhardt's Reagent, 100 µg denatured salmon sperm DNA at 42 °C and wash twice with 2X SSPE, 0.2% SDS at 42° C for 15 min each, then wash twice with 0.2X SSPE, 0.2% SDS at 55 °C for 30 min each;

(ii) the isolated nucleic acid fragment of (i) wherein the encoded polypeptide has an amino acid identity of 60% or greater to the polypeptide encoded by any one of the sequences set forth in SEQ ID NOS:1, 3, 5, 7, 9, 11, or 15; or

(iii) the isolated nucleic acid fragment of (i) wherein the nucleic acid identity is 90% or greater to any one of the sequences set forth in SEQ ID NOS:1, 3, 5, 7, 9, 11, or 15;

(b) growing fertile plants from seeds obtained from the cross; and

(c) screening progeny seeds from the fertile plants of step (b) for seeds containing reduced saturated fatty acids.

27. (amended) Oil obtained from the plants of Claims [21-26] 24-26.

Please add the following new claims:

28. A method of producing seed oil containing altered levels of unsaturated fatty acids comprising:

(a) transforming a plant oil-producing species with a chimeric gene comprising the nucleic acid fragment encoding all or a portion of a plant delta-12 desaturase;

(b) growing fertile plants from the transformed plant cells of step (a);

(c) screening progeny seeds from the fertile plants of step (b) for the desired levels of unsaturated fatty acids;

(d) processing the progeny seed of step (c) to obtain seed oil containing altered levels of unsaturated fatty acids.

29. The product of the method of Claim 28.

30. Canola oil obtained from the seeds of a plant having a chimeric gene capable of causing altered levels of fatty acid in a transformed canola plant cell, said chimeric gene comprising an isolated nucleic acid fragment comprising a sequence encoding a fatty acid desaturase with an amino acid identity of 50% or greater to the polypeptide encoded by any one of the sequences set forth in SEQ ID NOS: 1, 3, 5, 7, 9, 11 or 15, operably linked to a suitable regulatory sequence.

31. The soybean oil of Claim 9 obtained from the seeds of a plant having a chimeric gene capable of causing altered levels of fatty acid in a transformed soybean plant cell, said chimeric gene comprising an isolated nucleic acid fragment comprising a sequence encoding a fatty acid desaturase wherein the amino acid identity is 60% or greater to the polypeptide encoded by any one of the sequences set forth in SEQ ID NOS: 1, 3, 5, 7, 9, 11 or 15, operably linked to a suitable regulatory sequence.

32. The canola oil of Claim 30 obtained from the seeds of a plant having a chimeric gene capable of causing altered levels of fatty acid in a transformed canola plant cell, said chimeric gene comprising an isolated nucleic acid fragment comprising a sequence encoding a fatty acid desaturase wherein the amino acid identity is 60% or greater to the polypeptide encoded by any one of the sequences set forth in SEQ ID NOS: 1, 3, 5, 7, 9, 11 or 15, operably linked to a suitable regulatory sequence.

33. An isolated nucleic acid fragment comprising a nucleic acid sequence selected from the group consisting of:

(a) a nucleic acid sequence encoding a fatty acid desaturase or a fatty acid desaturase-related plant enzyme with an amino acid identity of 50% or greater to the polypeptide encoded by any one of the sequences set forth in SEQ ID NOS: 1, 3, 5, 7, 9, 11, or 15, or

(b) a nucleic acid sequence or a part thereof which is useful in antisense inhibition or sense suppression of endogenous desaturase activity in a transformed plant wherein the nucleic acid has an identity of 80% or greater to any one of the sequences set forth in SEQ ID NOS: 1, 3, 5, 7, 9, 11, or 15 or a part thereof.

34. The isolated nucleic acid fragment of Claim 33 wherein the amino acid identity is 60% or greater to the polypeptide encoded by any one of the sequences set forth in SEQ ID NOS:1, 3, 5, 7, 9, 11, or 15.

35. The isolated nucleic acid fragment of Claim 33 wherein the nucleic acid identity is 90% or greater to any one of the sequences set forth in SEQ ID NOS:1, 3, 5, 7, 9, 11, or 15.

36. The isolated nucleic acid fragment of any of Claim 33, 34 or 35 wherein said fragment is isolated from an oil-producing plant species.

37. A chimeric gene comprising the nucleic acid fragment of Claim 33, 34 or 35 operably linked to suitable regulatory sequences.

38. A plant comprising in its genome the chimeric gene of Claim 37.

39. Seeds obtained from the plant of Claim 38.

40. A method of producing seed oil containing altered levels of unsaturated fatty acids comprising:

- (a) transforming a plant cell of an oil-producing species with a chimeric gene of Claim 37;
- (b) growing fertile plants from the transformed plant cells of step (a);
- (c) screening progeny seeds from the fertile plants of step (b) for the desired levels of unsaturated fatty acids; and
- (d) processing the progeny seed of step (c) to obtain seed oil containing altered levels of unsaturated fatty acids.

41. The isolated nucleic acid fragment of Claim 33, 34, or 35 comprising a nucleic acid sequence encoding a plant microsomal delta-12 fatty acid desaturase.

42. A nucleic acid fragment isolated from an oil-producing plant species wherein said fragment is selected from the group consisting of:

- (i) an isolated nucleic acid fragment comprising a nucleic acid sequence encoding an enzyme which catalyzes a reaction at carbon positions 6 and 7 numbered

from the methyl end of an 18 carbon long fatty acyl chain, wherein positions 6 and 7 correspond to carbon positions 12 and 13 numbered from the carbonyl carbon of an 18 carbon long fatty acyl chain and further wherein the amino acid sequence comprising said enzyme contains at least one of the following amino acid sequences selected from the group consisting of: AIPPHCF, AWXXYW, HECGH, LLVPY, WKYSHR, and SHRRHH;

(ii) an isolated nucleic acid fragment encoding an enzyme which catalyzes a reaction at carbon positions 6 and 7 numbered from the methyl end of an 18 carbon long fatty acyl chain wherein positions 6 and 7 correspond to carbon positions 12 and 13 numbered from the carbonyl carbon of an 18 carbon long fatty acyl chain wherein said isolated nucleic acid fragment encodes a protein comprising any one of the amino acid sequences set forth in SEQ ID NOS:2, 4, 6, 8, 10 or 12;

(iii) an isolated nucleic acid fragment encoding an enzyme which catalyzes a reaction at carbon positions 6 and 7 numbered from the methyl end of an 18 carbon long fatty acyl chain, wherein positions 6 and 7 correspond to carbon positions 12 and 13 numbered from the carbonyl carbon of an 18 carbon long fatty acyl chain wherein said isolated nucleic acid fragment hybridizes to the isolated nucleic acid fragment of (ii) under one of the following sets of conditions:

(a) hybridization in 50 mM Tris, pH 7.6, 6X SSC, 5X Denhardt's, 0.5% sodium dodecyl sulfate (SDS), 100 µg denatured calf thymus DNA and 50°C and wash twice with 2X SSC, 0.5% SDS at room temperature for 15 min each, then wash twice with 0.2X SSC, 0.5% SDS at room temperature for 15 min each and then wash twice with 0.2X SSC, 0.5% SDS at 50 °C for 15 min each;

(b) hybridization in 6X SSPE, 5X Denhardt's solution, 0.5% sodium dodecyl sulfate (SDS), 5% dextran sulfate, 100 µg denatured calf thymus DNA at 50°C and wash twice with 2X SSC, 0.5% SDS at room temperature for 15 min each, then wash twice with 0.2X SSC, 0.5% SDS at room temperature for 15 min each and then wash twice with 0.2X SSC, 0.5% SDS at 50 °C for 15 min each; or

(c) hybridization in 50% formamide, 5X SSPE, 1% sodium dodecyl sulfate (SDS), 1% Denhardt's Reagent, 100 µg denatured salmon sperm DNA at 42 °C and wash twice with 2X SSPE, 0.2% SDS at 42° C for 15 min each, then wash twice with 0.2X SSPE, 0.2% SDS at 55 °C for 30 min each

43. A chimeric gene comprising the nucleic acid fragment of Claim 42 operably linked to suitable regulatory sequences.

44. A plant comprising in its genome the chimeric gene of Claim 43.



45. A method of producing seed oil containing altered levels of unsaturated fatty acids comprising:

- (a) transforming a plant cell of an oil-producing species with a chimeric gene of Claim 43;
- (b) growing fertile plants from the transformed plant cells of step (a);
- (c) screening progeny seeds from the fertile plants of step (b) for the desired levels of unsaturated fatty acids; and
- (d) processing the progeny seed of step (c) to obtain seed oil containing altered levels of unsaturated fatty acids.

46. A method for altering fatty acids composition in seeds comprising:

- (a) making a cross between a mutant line with altered fatty acid composition with a plant containing the chimeric gene of Claim 37;
- (b) growing fertile plants from seeds obtained from the cross; and
- (c) screening progeny seeds from the fertile plants of step (b) for seeds containing altered fatty acid levels.

47. A method for altering fatty acids composition in seeds comprising:

- (a) making a cross between a mutant line with altered fatty acid composition with a plant containing the chimeric gene of Claim 43;
- (b) growing fertile plants from seeds obtained from the cross; and
- (c) screening progeny seeds from the fertile plants of step (b) for seeds containing altered fatty acid levels.

48. A method for reducing polyunsaturated fatty acids in rapeseed oil comprising:

- (a) making a cross between a rapeseed variety with increased oleic acid content or reduced linolenic acid content with a plant containing the chimeric gene of Claim 37;
- (b) growing fertile plants from seeds obtained from the cross; and
- (c) screening progeny seeds from the fertile plants of step (b) for seeds containing reduced polyunsaturated fatty acids.

49. A method for reducing polyunsaturated fatty acids in rapeseed oil comprising:

(a) making a cross between a rapeseed variety with increased oleic acid content or reduced linolenic acid content with a plant containing the chimeric gene of Claim 43;

(b) growing fertile plants from seeds obtained from the cross; and

(c) screening progeny seeds from the fertile plants of step (b) for seeds containing reduced polyunsaturated fatty acids.

50. The method of Claim 46 or 47 wherein the cross in (a) is between a progeny plant derived from a seed comprising a Brassica variety having an oleic acid content of about 69% to 77%, based upon total extractable oil and belonging to a line in which the said oleic acid content has been stabilized for both the generation to which the seed belongs and its parent generation.

51. A method for reducing saturated fatty acids in rapeseed seeds comprising:

(a) making a cross between a rapeseed variety with increased oleic acid content with a plant containing the chimeric gene of Claim 37;

(b) growing fertile plants from seeds obtained from the cross; and

(c) screening progeny seeds from the fertile plants of step (b) for seeds containing reduced saturated fatty acids.

52. A method for reducing saturated fatty acids in rapeseed seeds comprising:

(a) making a cross between a rapeseed variety with increased oleic acid content with a plant containing the chimeric gene of Claim 49;

(b) growing fertile plants from seeds obtained from the cross; and

(c) screening progeny seeds from the fertile plants of step (b) for seeds containing reduced saturated fatty acids.

53. A method for reducing polyunsaturated fatty acids in soybean oil comprising:

(a) making a cross between a soybean variety with increased oleic acid content or reduced linolenic acid content with a plant containing the chimeric gene of Claim 37;

(b) growing fertile plants from seeds obtained from the cross; and

(c) screening progeny seeds from the fertile plants of step (b) for seeds containing reduced polyunsaturated fatty acids.

54. A method for reducing polyunsaturated fatty acids in soybean oil comprising: